Original Article

Association of serum Vitamin A, calcium, alkaline phosphatase, and phosphorus levels with recurrent acute lower respiratory infections among children

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ABSTRACT

Introduction: Acute lower respiratory infection (ALRI) is a leading cause of morbidity and mortality in children younger than 5 years of age all over the world especially in developing countries. Consequently, considerable research has been aimed at finding possible risk factors in ALRI; micronutrients have been considered one of them. **Materials and Methods:** The present study was conducted to determine any association of serum Vitamin A, beta-carotene, calcium, alkaline phosphatase, and phosphorus levels with recurrent ALRI in children <5 years of age. A prospective hospital-based case-control study was planned for a period of 10 months at a tertiary care referral center in central India. **Results:** A total number of 85 children aged 6 months to 5 years were randomly enrolled in two groups as case, i.e., recurrent ALRI (n=40) and control (n=45). Levels of serum Vitamin A and beta-carotene were found significantly lower in children with recurrent ALRI than in the control group ($p<0.05$). However, serum levels of calcium, alkaline phosphatase, and phosphorus did not differ significantly when compared with control group ($p>0.05$). **Conclusion:** The results provide evidence for the role of Vitamin A and beta-carotene levels in recurrent ALRI in pediatric patients. Our study was not able to find a significant relationship between calcium, phosphorus, and alkaline phosphatase levels with the occurrence of recurrent ALRI.

Key words: *Acute lower respiratory infection, Alkaline phosphatase, Calcium, Phosphorus, Vitamin A*

Acute respiratory infections are very common in children. They are classified as upper respiratory tract infections and lower respiratory tract infections. Acute lower respiratory infection (ALRI) refers to the acute inflammation of the pulmonary tissues or airways below the level of the larynx, common ones being pneumonia, bronchiolitis, empyema, lung abscess, and bronchiectasis. The incidence of ALRI under 5 years age group is estimated to be 0.29 episodes and 0.05 episodes per child-year in developing and in developed countries, respectively [1]. This translates into about 156 million new episodes each year worldwide, of which 151 million episodes are in the developing countries. India, being a developing country, contributes most with 43 million cases.

Of all infectious disorders, pneumonia, diarrhea, and malaria were the leading causes of death worldwide in under five children. Every year approximately 1.396 million deaths (18.3% of total deaths) are caused due to pneumonia worldwide [2]. In India, according to a study, 0.38 million children were died in 2010 due to pneumonia [3]. The risk factors which are found to be associated with pneumonia are lower socioeconomic status, severe malnutrition, lack of breastfeeding and similar illness in other family members.

Considerable research has been aimed at finding effective interventions such as immunization and administering antibiotics [4]. Micronutrient supplementation is another area of potential research and intervention, for example, zinc, Vitamin A, and Vitamin D [5-7]. One such micronutrient is phosphorus. Research showed that hypophosphatemia leads to impaired chemotaxis, phagocytosis, and bactericidal activity of macrophages [8]. In addition, selected cases of atypical pneumonia have been shown to be associated with hypophosphatemia [9]. Studies were done in adult patients suggest a possible association between hypophosphatemia and the development of pneumonia, suggesting that these patients were more prone for severe infection secondary to their hypophosphatemia [10]. Fat-soluble vitamins and beta-carotene are the major chain-breaking antioxidants in body tissues and are considered to be the first line of defense against lipid peroxidation, protecting cell membranes at an early stage through free radical-quenching activity [11,12].

Therefore, the present study was undertaken to explore any association of serum Vitamin A, beta-carotene, calcium, alkaline phosphatase, and phosphorus levels with recurrent ALRI in pediatric age groups.

MATERIALS AND METHODS

This hospital-based case-control study was conducted in the Department of Paediatrics, M.G.M. Medical College and M.Y. Hospital, Indore, a tertiary care referral center in MP. The study duration was of 10 months (July 2016 to May 2017). Case and

control groups were selected according to following criteria and randomized in appropriate groups. The study protocol was approved by Institutional Ethics Committee, and informed consent was obtained from the parents or guardians of all participants before the study after explaining the study protocol.

Inclusion Criteria

Children between 6 months and 5 years of age admitted with recurrent ALRI during the study period were included as cases. A case definition of severe ALRI as given by the World Health Organization-1995 was used [13]. Recurrence of pneumonia was defined as two or more episodes in a single year or three or more episodes ever with radiographic clearing between occurrences [14]. Healthy children attending the vaccination clinic living in the same area during the study period between 6 months and 5 years of age were selected as controls. Biochemical estimations were done in control group before administration of routine doses of Vitamin A.

Exclusion Criteria

Patients were excluded from the study if they had evidence of disorders such as congenital heart disease, asthma, protein energy malnutrition (PEM) Grades III and IV (according to International Association of Paediatrics classification of malnutrition) [15], clinical signs and symptoms of Vitamin A and Vitamin D deficiency, and evidence of chronic illnesses (such as chronic kidney disease, chronic liver disease, tuberculosis, cerebral palsy, gastroesophageal reflux disease, and seizure disorder) as these conditions have been shown to be independent risk factors for ALRI.

For infants and children who satisfied the study criteria, following information was recorded: Age, sex, anthropometric measurements, and duration of hospital stay, complete blood count, and chest X-ray. On the 1st day of each admission, venous blood specimens (5 ml) were collected from the children enrolled either in the case or control groups and placed in a test tube covered with a dark brown paper for serum biochemical analysis. The serum levels of Vitamin A were determined according to the method of Suzuki and Katoh using double beam Ultraviolet spectrophotometer (Shimadzu-1700, Japan). Briefly, serum (1 ml) was mixed with ethanol (1 ml) and hexane (3 ml) and the tube was shaken mechanically for 10 min, and all tubes were then centrifuged at about 800× g for 10 min. After that, hexane extract was spectrophotometrically evaluated at absorbance maxima at 325 nm and 453 nm for estimation of Vitamin A and beta-carotene, respectively [16]. The serum levels of calcium, alkaline phosphatase, and phosphorus were analyzed on autoanalyzer (Biosystem-A15, Spain) using commercial reagent kits.

All data were expressed as the mean±standard error mean. Statistical analysis (Student's t-test) was evaluated by Prism statistical software program, and values of patients with ALRI (case) were compared to those of control group and differences at the level of 5% ($p<0.05$) were considered to be statistically significant.

RESULTS

Over a period of 10 months, 368 infants and children were admitted to pediatric wards with ALRI, contributing to 12% of total admissions in our Paediatric Department. Out of 368 children admitted with ALRI during the study period, 40 (26 male and 14 female) children fulfilled the inclusion criteria for the study and 45 (27 male and 18 female) age-matched healthy children from immunization clinic were taken as controls. Males outnumbered females in both case and control group as male to female ratio was found to be 1.5.

The mean age of all the children was 23.9±15 months. Of the 85 participants, 33 (39%) were between 6 and 12 months, 34 (40%) were 12–36 months, and 18 (21%) were 48–60 months; there was no significant difference between cases and controls in age mean or distribution. Among ALRI cases, maximum number, i.e., 19 (47.7%) were of age group 6 months to 1 year, 14 (35%) of age group 1–3 years, and 7 (17.5%) were between the age of 3 and 5 years. Most of the children, i.e., 69.5% were resident of the urban area. Almost half, i.e., 42 of children were well nourished, 27 (31.7%) were of PEM Grade I, and rest 16 (18.8%) were of PEM Grade II (Table 1).

Majority of children in the case group had pneumonia, i.e., 60% and remaining had bronchiolitis (35%) and empyema (5%). Among those 24 cases of pneumonia, 6 were of age group 6 months–1 year, 10 cases of 1–3 years and 8 cases were between 3 and 5 years of age group (Table 2). Among the cases, duration of hospital stay was <3 days in 9 (22%) cases, 3 days to 1 week in 17 (43%) cases, 1–2 weeks in 13 (22%) cases, and more than 2 weeks in only one case.

Mean serum calcium levels were found to be lower in the case group, i.e., 7.935 mg/dl than in the control group, i.e., 7.991 mg/dl, but these results were not found statistically significant. Similarly, mean phosphorus level in the control group was 5.942 mg/dl and in the case group was 5.872 mg/dl, results were not statistically significant. The mean level of alkaline phosphatase in the case group was 302.6 U/L and in the control group was 291.2 U/L. Hence, values were higher in cases than in control, but statistically, it was insignificant (Table 3).

Mean serum level of Vitamin A in children with recurrent ALRI and the control group was found to be 45.58 µg/dl and 78.48 µg/dl, respectively. While beta-carotene level in the case and in the control groups were 34.87 µg/dl and 51.92 µg/dl, respectively. Thus, it is clearly observed that the serum levels of Vitamin A and beta-carotene decreased in recurrent ALRI as compare to the control. The results showed a statistically significant difference between the case and control groups ($p<0.05$). Clinical observations of the study also revealed the prevalence of sub-clinical Vitamin A deficiency in children with recurrent ALRI (Table 4).

DISCUSSION

In the present study, ALRI contributed to 12% of admissions in the Paediatric Department. Similarly, in a study conducted by Savitha *et al*., infants constituted 62.5% of ALRI cases. Studies were done by Cunha *et al*. reported that age <1 year was a risk factor for

Table 1: Social profile of cases and controls

Variables	Cases n=40 (%)	Control n=45 (%)	Total 85 (%)
Age (years)			
6 months-1	19 (47.5)	14 (31.2)	33 (38.8)
1–3	14 (35)	20 (44.4)	34 (40)
3–5	7 (17.5)	11 (24.4)	18 (21.2)
Gender			
Males	23 (57.5)	28 (62.2)	51 (60)
Females	17 (42.5)	17 (37.8)	34 (40)
Place			
Rural	15 (37.5)	11 (24)	26 (30.5)
Urban	25 (62.5)	34 (76)	59 (69.5)
Nutritional status			
Normal	22 (55)	20 (44.5)	42 (49.4)
PEM Grade I	10 (25)	17 (37.7)	27 (31.7)
PEM Grade II	8 (20)	8 (17.7)	16 (18.8)

PEM: Protein-energy malnutrition

Table 2: Disease wise distribution of ALRI cases

Age (years)	Pneumonia (%)	Bronchiolitis (%)	Empyema (%)	Total (%)
6 months-1	6 (25)	10 (71.4)	0 (0)	19
1–3	10 (41.6)	4 (28.6)	1 (50)	14
3–5	8 (33.4)	0 (0)	1 (50)	7
Total	24 (60)	14 (35)	2 (5)	40

ALRI: Acute lower respiratory infection

Table 3: Laboratory values of case and control groups

Laboratory investigation	Cases (%)	Control (%)	Total (%)
Serum retinol (µg/dl)			
<10	6 (15)	2 (4)	8 (9)
10–20	7 (18)	2 (4)	9 (11)
More than 20	27 (67)	41 (92)	68 (80)
Serum calcium (mg/dl)			
<8.8	31 (77)	37 (82)	68 (80)
More than 8.8	9 (23)	8 (18)	17 (20)
Serum phosphorus (mg/dl)			
<2.5	11 (28)	3 (7)	14 (16)
More than 2.5	29 (72)	42 (93)	71 (84)
Alkaline phosphatase			
<800	12 (30)	5 (12)	17 (20)
More than 800	28 (70)	40 (88)	68 (80)

respiratory morbidity [17]. In the present study, ALRI was found to be more common among infants (48%) [18]. The present study supports the findings of other studies done in Ankara in 1990 and Micronesia in 1993 that subclinical Vitamin A deficiency was found significantly in children with ALRI [19,20].

The similar result was found in a study done recently in China on children <3 years of age. It showed that children with pneumonia often had low levels of Vitamin A. The level of Vitamin A is associated with the severity of pneumonia and the development of recurrent respiratory infection afterward [21]. Vitamin A plays a key role in the development and function of virtually all cells, not just those of epithelium and endothelium. It costimulates T cells through CD3 and is involved in T-cell maturation, differentiation, and proliferation [22-24]. Vitamin A and beta-carotene (a precursor of Vitamin A) have anti-oxidant potential and can function as effective radical-trapping antioxidants.

There is ample evidence that beta-carotene and Vitamin A are very effective quenchers of singlet oxygen and play a key role in the prevention of diseases [11,12]. Thus, deficiency of Vitamin A and beta-carotene may reduce immunity and contributes in causing recurrent ALRI and may be associated with pulmonary and systemic symptoms of recurrent ALRI. A large study done in Ethiopia by Muhe *et al*. found that Vitamin D and calcium

Table 4: Comparison of serum Vitamin A, beta-carotene, calcium, alkaline phosphatase and phosphorus levels with recurrent ALRI

Laboratory tests	Mean±SD	Z	p
Serum retinol (mg/dl)			
Case	45.58±41.205	2.98	<0.05
Control	78.48±60.122		
Beta-carotene (mg/dl)			
Case	34.87±19.96	3.297	<0.05
Control	51.92±27.53		
Serum calcium (mg/dl)			
Case	7.935±1.0118	0.2798	>0.05
Control	7.991±0.818		
Serum phosphorus (mg/dl)			
Case	5.872±1.722	0.215	>0.05
Control	5.942±1.274		
Alkaline phosphatase (U/L)			
Case	302.6±135.9	0.234	>0.05
Control	291.2±292.3		

SD: Standard deviation

deficiency were more common in children with pneumonia than in normal children. It was proposed that Vitamin D or calcium deficiency may be important predisposing factors for pneumonia in children aged under 5 years [25].

The bio-chemical investigational data of calcium and alkaline phosphatase in our study were not found to be changed significantly in case and control groups ($p>0.05$). Although the serum phosphorus levels were found to be slightly lower in cases (5.872±0.272 mg/dl) than in the control group (5.942±0.190 mg/dl), this difference was not found to be statistically significant ($p>0.05$). Similar results were shown in a study done on Nigerian children with pneumonia between 2 months and 5 years of age [26]. The difference in mean serum levels of calcium, phosphorus, and alkaline phosphatase was not found to be statistically significant. A study was done by Gharehbaghi *et al*. in Turkey compared the same in neonates with ALRI with healthy neonates [27]. They also found no statistical difference. Hence, serum calcium, phosphorus, and alkaline phosphatase level do not seem to make infants and children prone to ALRI.

Limitation of our study was the small number of the subjects; therefore, further prospective trials with a large number of subjects and for a longer duration are advised.

CONCLUSION

Our study showed that lower levels of Vitamin A may be associated with recurrent ALRI in pediatric patients. The results of this study have important public health implications since, irrespective of the routine doses of Vitamin A; children are deficient in Vitamin A. We suggest strengthening supplementation of Vitamin A doses with routine immunization that would help in reducing the attack of ALRI and may be benefited from prophylactic Vitamin A doses. However, additional studies are needed to substantiate these suggestions.

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